[Proc. Roy. Soc. Victoria, 53 (N.S.), Pt. I., 1941.]

Art. V.—*A Bacterial Disease of Stocks caused by* Phytomonas matthiolae.

By ROSE MUSHIN, B.Sc.

[Read 13th June, 1940; issued separately 1st February, 1941.]

Introduction.

Early in September 1938, a disease of stocks (*Matthiola incana* R. Br. var. *annua* Voss.) was reported from Brunswick Park, Victoria. The stocks showed a high percentage of diseased plants distributed in large patches amongst the healthy ones. Enquiries revealed that the disease became evident in August and with the warmer weather conditions did not spread to neighbouring plants.

The bacteria causing diseases of stock can be divided into two groups. One comprises *Bact. campestre* (Pammel) E. F. Smith and bacteria closely related to it as recorded by von Faber (13) in 1907, Cooley (12) in 1932, Kendrick (17) in 1938 and by Wilson (30) in New South Wales in 1938. The organism described in this work belongs, however, to a second group of green fluorescent bacteria.

Briosi and Pavarino (4) in 1912 first reported a green fluorescent organism causing a disease of stocks in Italy and named it *B. matthiolae*. They described the symptoms and the morbid anatomy of the attacked plants, but no critical account of the organism was given. Rudorf and Job (24) in 1932 observed a similar disease and on the basis of external symptoms and histological studies concluded it to be caused by *B. matthiolae*. Adam and Pugsley (1) in Victoria in 1934 recorded the occurrence of a disease of stocks due to a green fluorescent bacterium but thought it distinct from the one reported in Italy. In 1937 a disease of stocks was observed on the Italian Riviera (23) reported to be caused by *Bact. matthiolae*. Burkholder (8) suggested that *B. matthiolae* is identical with *Phytomonas syringae*, as inoculations of stock plants with the latter organism produced symptoms similar to these described by Briosi and Pavarino and by Adam and Pugsley.

Observing that no detailed account of the organisms described by the above-mentioned writers was supplied, it seemed advisable to make a study of the disease brought under notice.

Symptoms.

The diseased stocks observed in the field in the earlier or milder stage of the attack showed light green spots on the leaves. At a later stage the leaves became discoloured, pale green, with

scattered dark green spots and an uneven surface presenting a puckered appearance. Badly affected plants were dwarfed, the leaves small and deformed with incurled margins, the flowers discoloured and under-developed. In some instances it was noticed that the stems became woody and new lateral shoots sprang up which were stunted, carried deformed leaves and flowered poorly. Some stems showed dark brown streaks or splits and the cortex was discoloured. To the naked eye the roots appeared normally developed.

Isolation.

In the early stages of the disease the plants often yielded a pure culture of the pathogen, while badly infected plants usually showed invasion by secondary parasites. The infection being systemic, isolations were made from any part of the stem or leaves and pure cultures were used for inoculation tests.

Inoculation Studies.

To test the pathogenicity of the organism isolated, 24 hr. cultures on agar slopes emulsified with a few c.c. of sterile water were used for inoculations. A few sets of inoculation experiments were performed. In the first set in September, 1938, three horticultural varieties of stocks were used: Imperial, Ten Weeks and Nice. The last named proved to be most susceptible to infection. Seedlings were inoculated, the procedure adopted being to place them in moist chambers for 24 hours before and after the inoculation. Out of six stock plants of Nice variety two were treated as control. Two inoculated by puncturing the stem developed local necrotic lesions, causing splitting of the cortex and stunting in growth. Two others were inoculated by pricking the leaves with a charged needle and one of them displayed within one week light green roundish spots about 2 mm. in diameter, followed by puckering of the leaves while the other showed only small brownish lesions around points of inoculation.

Later inoculation tests were made with pure cultures re-isolated from the artificially infected stocks. These tests were performed on plants of different ages and under varied conditions by puncturing the stem or leaves with a charged needle or by spraying. In most cases infection was obtained but the symptom picture was different, probably due to temperature and humidity changes, as no facilities for maintaining constant environmental conditions were available. Evidence pointed to the conclusion that young seedlings kept under lower temperatures were most badly affected. Sometimes only local brownish lesions were produced on stems and leaves, these later becoming puckered. In other cases irregular light green spots appeared on the leaves not only around points of inoculation but scattered irregularly. Dwarfing and deformation of leaves was produced most often by inoculating the stems of young seedlings. Hand cut and

microtome sections of the infected stocks showed the presence of bacteria. Numerous re-isolations of the organism were carried out and the green fluorescent bacterium was identified by means of cultural and biochemical characters.

Morbid Anatomy.

Plants which had been infected both naturally and experimentally were used for the purpose of obtaining material for hand-cut and microtome sections. Staining of the preserved tissues with Loeffler's methylene blue for 10-15 mins. and subsequent decolourisation with 0.5 per cent. acetic acid gave satisfactory results. The bacteria were found in the parenchymatic cells and the vascular vessels, which were often blocked either by bacteria or a brown exudate. In advanced stages of the disease the tissues showed cavities filled with bacteria. The roots were less susceptible to invasion than the aerial parts of the plants.

Cross Inoculations.

Smith (28) considered the Italian stock disease caused by *B. matthiolae* to be similar to the Dutch disease of wallflower, of which no complete account was given. Burkholder (8) pointed out the similarity in the description of the stock organism and *P. syringae*. A survey of literature indicated the desirability of experimental inoculation of tobacco and tomato as some other members of the genus Phytomonas (Burkholder (6), (9)) are able to infect plants of the Solanaceae and Cruciferae families, producing leaf spots, e.g. *P. tabacum* (Wolf and Foster (16)) or *P. vesicatorum* var. *raphani* (White (29)), resembling *P. campestre*. Also simultaneous biochemical studies proved the stock organism to be similar in its characters to *P. polycolor* (11), causing leaf spot on tobacco (Clara (10)) and to *P. marginale* (11), infecting lettuce (Brown (3)).

Cross inoculations with the organism re-isolated from the experimentally infected stocks were made to wallflower, lettuce, lemon fruit, tobacco and tomato plants. The method of cross-inoculation was similar to the one applied for infecting stocks. The results are given in *Table* 1.

Table 1.

Type of Plant.	Cross Inoculations.
Lemon fruit	No symptoms of infection.
Wallflower	Stem inoculations produced brown necrotic lesions; leaf punctures caused puckering and sometimes a slight deformity.
Lettuce and Tomato	Stem and leaf inoculations produced small brownish lesions around points of inoculation.
Tobacco	Stem inoculations caused slight necrotic lesions; around the leaf punctures lighter coloured zones were noticeable.

Attempts to re-isolate the stock organism from lemon fruit were unsuccessful. Isolations made 10 days after inoculations from the edge of lesions of wallflower, lettuce, tomato and tobacco plants resulted in obtaining cultures of the green fluorescent bacterium, identical with the one used for inoculations. No dwarfing or deformity of plants was observed at any later date. Isolations made from parts of the plants at some distance from the points of inoculation did not yield the bacterium.

These experiments indicated that the organism could survive in the cells of the host plants mentioned but was not able to produce systemic infection.

The Causal Organism.

Pure Culture Studies.

The technique adopted—unless otherwise stated—was in accordance with the methods recommended by the Manual of Methods for Pure Culture Study of Bacteria (Society of American Bacteriologists).

Morphology.

The morphology and size of the cells was obtained by observing smears treated by Benian's method with congo red. The cultures were grown on beef extract agar of pH 6.8 for 24 hours at 24°C. A colony on agar plate of the organism freshly isolated from stock consisted of cocco-bacilli of 0.75 x 1.0μ in size, or small rods with rounded ends whose length did not exceed 1.8μ. The bacteria occurred singly or in pairs. A smear from an agar slope culture showed rods with rounded ends, some straight, others slightly bent 0.75–1.1μ x 1.3–3μ in size. The cells were distributed singly or in pairs. Older laboratory cultures consisted usually of longer rods than the younger ones and often had chains 7μ to 35μ in length, in which the division into cells was not clear.

Bacteria from a 24 hour old broth culture under dark ground illumination appeared as cylindrical rods, many paired together, some in short chains, displaying a swift darting movement across the field. Flagella were demonstrated by staining the smears from a 24 hour old agar culture by Cesares-Gil's method, using the mordant in 1 : 1 dilution and carbol fuchsin for 5 minutes. The flagella were polar, one or two in number. A few cells showed bi-polar flagella having one flagellum at each end of the cell or one at one end and two at the other; these cells might have been in the process of division as a slight constriction in the middle was noted. The flagella were wavy and longer than the cells.

The bacteria from a 24 hour old agar growth stained evenly using a simple stain, while in older cultures some cells stained deeper in contrast to other faintly stained ones. The organism proved to be Gram negative, not acid fast and non-sporing. No capsules were demonstrated by the methods of Hiss or Anthony.

Cultural Characters.

All cultures were incubated at 24°C.

Agar Colonies.—A 24 hour old agar plate of the freshly isolated organism displayed punctiform and circular colonies 1-2 mm. in diameter. Some reached 4 mm. after a few days of incubation. The colonies were smooth with an entire edge, raised and translucent, showing a brownish tint in transmitted light. The plates had an unpleasant odour. A poured plate from a laboratory culture showed after two weeks of incubation circular colonies 6-7 mm. in diameter with a greyish inner circle 5 mm. in diameter, containing a thick point in the centre and a translucent and striated ring outside the circle. Amongst the colonies some were found with a lobated edge, rather flat, translucent, often possessing an outer striated ring.

Agar Stroke.—An abundant growth appeared after 24 hours of incubation, filiform with a slightly undulate margin, glistening, of butyrous consistency, producing a greenish colouring of the medium. After three days the green colouring became more distinct and the edge of the growth looked like a fine scalloped trimming. Later the medium turned light brown. Older laboratory cultures exhibited a less distinct green pigment which became more intense on rejuvenating the bacteria by passage through broth and through a few quick successive transfers to fresh agar slopes. The freshly isolated cultures had an unpleasant odour which became less offensive with age.

Agar Stab.—Growth occurred along the line of inoculation reaching the bottom of the tube.

Nutrient Broth.—Twenty-four hour old cultures showed strong turbidity. Clouds were raised on shaking. No pellicle or sediment was visible. After 48 hours the clouding became deeper, so that print could not be read through it. At the surface of the cultures, at points of adhesion to the walls, a ring of granulated growth was noticed. In a few days a creamy sediment settled on the bottom of the tube while on the surface a very delicate pellicle was formed, easily detached by a slight disturbance. Often the surface displayed no pellicle, only a ring of granulated growth. A green fluorescent zone appeared at the upper part of the broth and gradually diffused into the medium.

Gelatin Stab.—At 20°C. in 24 hours the growth was best at the surface, the line of puncture being filiform with an initial drop of liquefaction, starting at top. In four days the liquefaction became infundibuliform and spread to three-quarters of the medium. The liquefied area took on a green colouring, displaying a pellicle and a sediment. In six days the gelatin was liquefied from wall to wall reaching almost to the bottom of the tube.

Milk.—In 24 hours a ring of digestion appeared at the surface. In five days one-third of the medium was digested and became greenish. In eleven days half of the medium was watery,

greenish, and turned alkaline, while a soft creamy coagulate settled down. In twenty days the medium was cleared, greenish with a yellowish coagulate down the tube.

Litmus Milk.—During the process of reduction of litmus many changes in colour took place, which were named according to Ridgway's classification. In the course of many observations it became evident though, that the occurrence of different shades was not constant, and in consequence a crude naming of the colours seemed most reasonable.

In 24 hours a slight ring of digestion appeared at the surface of the medium. In 48 hours a lower ring of discolorization became visible. In three days a soft coagulate started to form at the bottom of the tube. In five days one-third of the medium was digested and yellowish in transmitted light. In eight days this yellowish colour had a green tint. In twelve days the medium was digested and the reaction became alkaline. In one month in transmitted light the yellow digested medium had a distinct green tint, in two months it became brownish-red. In three months it took on a beautiful wine colour, while in reflected light it looked dull blackish-green, and the coagulate a dirty greenish colour.

Brom Cresol Purple Milk.—In 24 hours a zone of digestion appeared on the surface of the culture and progressed toward the bottom of the tube taking on a purplish tint. In eleven days the milk became distinctly purple in transmitted light; the medium was alkaline with a coagulate at the bottom of the tube.

Potato Medium.—An abundant moist light-brown growth was produced after 48 hours of incubation. The medium turned a darker shade.

Uschinsky's Solution.—Good growth, sediment and a firm pellicle. The medium turned brilliant green, starting with a zone at the surface.

Fermi's Solution (Tauner's variant).—Growth at first was less vigorous than in Uschinsky's solution, with a firm pellicle and sediment. Colouring was less distinct than in Uschinsky's medium.

Cohn's Solution.—No growth.

Sullivan's Solution.—(Clara (10)) showed constantly green fluorescence.

Physiological Characters.

Relation to Free Oxygen.—This was determined by growing the organism in agar shake cultures and on agar slopes under anaerobic conditions, when a scanty film of growth was formed. The bacterium proved to be a facultative anaerobe.

Action on Nitrates.—A reduction of nitrates to nitrites without gas production was observed in 24 hours. In 48 hours a complete

consumption of nitrate beyond the nitrite stage took place and ammonia was liberated. Parallel control tests were conducted with sterile nitrate broth media and *Escherichia coli* cultures.

Production of Ammonia.—For this test Hensen's (15) method was adopted. Ammonia production was detected in peptone broth cultures of pH 7.6 incubated for three days. A sterile medium was tested and proved to be ammonia free.

Indole Production.—Two and seven days' old peptone cultures were tested by the Ehrlich-Böhme technique. No indole was detected. *Esch. coli* cultures were used as controls.

Hydrogen Sulphide Production.—The lead acetate strip technique was adopted. No hydrogen sulphide was detected. *Esch. coli* cultures were used for comparison.

Hydrolysis of Starch.—Starch broth media were used according to Eckford's method. After ten days a partial hydrolysis took place, indicating the production of erythrodextrin. After six weeks of incubation the digestion of starch did not progress further, and no sugar was detected at any time with Fehling's solution.

Cellulose digestion was tested as described by Clara (10). None was recorded.

Utilization of Amino-acid Media.—The formula of Frazier and Rupp (14) was followed by adding different amino-acids to a basic mineral solution. Good growth was recorded in peptone, asparagin, and aspartic acid, weaker in glutamic acid and tyrosin, showing the ability of the organism to derive its carbon and nitrogen requirements from the above-mentioned amino-acids.

Carbohydrate Reactions.

Two kinds of media were prepared. One set contained media with a peptone base and an addition of 1 per cent. of various carbohydrates, sterilized by steaming on three successive days. One per cent of Andrade's indicator was added and the reaction was adjusted to pH 6.8. These media were provided as additional means of quick identification by biochemical reactions of the organism isolated from infected or artificially inoculated plants. The results are given in Table 2.

Table 2.

Carbohydrate reactions (in peptone media).

Glucose.	Galactose.	Lactose.	Sucrose.	Maltose.	Mannite.	Glycerin.	Salicin.
+	−	−	−	−	−	−	−

+ = acid. − = no acid, no gas.

Acid was recorded only in glucose and galactose after three days of incubation, while older laboratory cultures took a longer time to ferment these sugars.

The second set of media contained a peptone free base with an addition of various carbohydrates: sugars, alcohols, glucosides or organic acids. These media were made up by following Burkholder's (8) directions. To avoid any changes by heating of the media, all sugar solutions were passed through a Seitz filter. To record acid production brom cresol purple was used as an indicator, while phenol red was utilized to note any change to alkalinity in the organic acid media. The cultures of the stock organism used for inoculation of media were derived from the two morphologically different kinds of colonies found on agar plates, namely, the entire and the lobated edge types. Both of them gave identical biochemical reactions, recorded in the table below:—

TABLE 3.

Carbohydrate reactions (in peptone free media).

(1) *Acid produced* in:—
Rhamnose, glucose, levulose, galactose, mannose, glycerol, mannite, acetic acid, citric acid, formic acid, lactic acid, malic acid, succinic acid.

(2) *Feeble acid* production in:—
Maltose.

(3) *No acid, no gas* produced in:—
Lactose, sucrose, raffinose, starch, salicin, tartaric acid.

All fermented cultures displayed a distinct change of colour within six days of incubation, except rhamnose, which was more slowly fermented. Maltose was discoloured after three weeks, indicating only feeble fermentation.

TEMPERATURE RELATIONS.

Freshly inoculated agar and broth cultures were incubated at different temperatures and the influence on growth was noted.

TABLE 4.

Temperature Relations.

Temperature °C.	Growth	Colour of Medium.
0	Fair	No change
10	Fair	Sometimes slightly green
20–24	Abundant	Distinctly green
30	Good	No change
37	Slight	"
38·5	Little or none	"
41	None	"

Motility was most active in 24-hour-old broth cultures incubated at temperature between 10°C.–30°C. After three days the bacteria became sluggish. A pellicle or a granulated zone was

formed at the surface of broth cultures at temperatures between 10°C.–30°C., not observed at 0°C. or 37°C. It is interesting to note that cultures kept for a week at 0°C. or 37°C. and then transferred to an incubator at 24°C. or transfers made from cultures kept at 0°C. and 37°C. to media placed at 24°C. resulted in the production of an unusually brilliant green fluorescence.

Thermal Death Point.—This was determined by applying Magoon's capillary method. Nineteen-hour-old broth cultures were used for the test. 52°C. for ten minutes proved to be the thermal death point.

Relation to Reaction (pH) of Medium.—One loopful of a 24-hour broth culture was transferred to extract-beef broth media of different pH values, starting from pH 4.0 and reaching pH 10.0. On the acid side pH 4.4 proved to be the limit for growth, and pH 9.5 on the alkaline side. These limits of growth are in agreement with the ones estimated by Berridge (2). At these pH values the broth tubes showed only slight turbidity after 48 hours of incubation. Most distinct fluorescence of the medium took place at pH about 7.4 and seemed to be more pronounced on the alkaline side, still showing a diffused green colouring at pH 9.1. On the acid side pH 5.4 represented the limit line for the production of green fluorescence, and only a greenish zone was formed at the surface.

Effect of Desiccation.—This was determined by placing drops of 24-hour-old broth cultures on sterile coverslips and letting them dry (Smith (26)). No growth appeared after seven days of desiccation.

Effect of Direct Sunlight.—This was determined by following the method described by Smith (27). The test was carried out in the beginning of March about noon. Growth was obtained after an exposure of fifteen minutes, while no growth was recorded after an exposure of 30 minutes.

Vitality in Culture Media.—The organism was still alive in agar, milk, and gelatin cultures eight months' old, left at room temperatures. Eleven months' old agar and potato cultures were dry, and the bacteria proved to be dead.

Conclusions.

The described organism is considered to be identical with the one reported by Briosi and Pavarino (4), and named by them *Bacterium matthiolae*. The symptoms of plant infection, the inoculation tests, and the biochemical reactions of the organism prove it to be different to the one recorded by Adam and Pugsley (1) and not identical with *Phytomonas syringae* (8).

The organism belongs to the genus *Phytomonas* (6), so the proper name is *Phytomonas matthiolae*. A comparison with the green fluorescent bacteria listed by Clara (10) would include the

organism in the sub-group II. of the non-sucrose fermenters composed of soil saprophytes and weak plant parasites. This statement finds confirmation in the inability of the bacterium to attack all strains of stocks, and in the fact of the disease being checked easily by less suitable weather conditions.

The cultural and physiological characters of *P. matthiolae* show an affinity to *Pseudomonas fluorescens* (Flügge) Migula and to *P. aeruginosa* (Schröter) Migula (which is a synonym to *B. pyocyaneus* (Gossard)). Ruzicka in 1898 (25) considered *B. fluorescens* and *B. pyocyaneus* closely related, the difference in characters being due to the adaptation of *B. pyocyaneus* to a parasitic existence and of *B. fluorescens* to a saprophytic mode of living. Niederkorn (21) in 1900 stated that amongst the fluorescent bacteria only two constant forms are to be found, namely, *B. pyocyaneus* and *B. fluorescens*. With the advance of scientific work much data was collected indicating the close relationship of the green fluorescent bacteria (Burkholder (6), (9); Clara (10), (11); Lacey (18), (19)). It is interesting to note here that one of the non-sucrose fermenters *P. marginale* (Brown) was considered by Metha and Berridge (20) to be identical with *B. pyocyaneus*. In the light of recent research (22) thrown on the subject members of the *Phytomonas* group causing leaf-spot diseases are considered to be physiological adaptations of *Pseudomonas fluorescens*.

Short Description of *Phytomonas matthiolae*.

Small Gram −ve rod, occurring singly or in pairs, chains sometimes formed. Size 0.75 — 1.1μ × 1-3μ. No spores, no capsules. Agar colonies with entire or lobated edge. Growth on agar slope and in broth abundant, producing green fluorescence. Fragile pellicle in broth. Gelatin liquefied. Milk alkalinized, coagulate formed, litmus reduced. Growth with green fluorescence in Uschinsky's, Fermi's and Sullivan's solutions, none in Cohn's. Facultative anaerobe. Production of ammonia +ve, indol −ve, hydrogen sulphide −ve, cellulose digestion −ve, starch feebly hydrolyzed. Amongst peptone carbohydrate media glucose and galactose are fermented without gas production, while lactose, sucrose, maltose, mannite, glycerin and salicin are not fermented. Amongst peptone-free carbohydrate media rhamnose, glucose, levulose, galactose, mannose, glycerol and mannite also acetic, citric, formic, lactic, malic and succinic acid are fermented without gas production. No acid or gas is recorded in lactose, sucrose, raffinose, starch, salicin and tartaric acid. Maltose is feebly fermented. Optimum temperature 20-24°C., minimum below 0°C., maximum slightly above 38.5°C. Thermal death point 52°C. for 10 minutes. Limits of growth in broth are pH 4.4 to pH 9.5. Best fluorescence at optimum temperature at about pH 7.4. The limit for desiccation is 7 days, for direct sunlight exposure 30 mins.

Summary.

An investigation was conducted on a disease of stocks which was reported in Victoria in 1938. The disease was proved to be caused by *Phytomonas matthiolae* originally recorded by Briosi and Pavarino in Italy, but not observed hitherto in Australia. Artificial infections were successful with healthy stocks of Nice

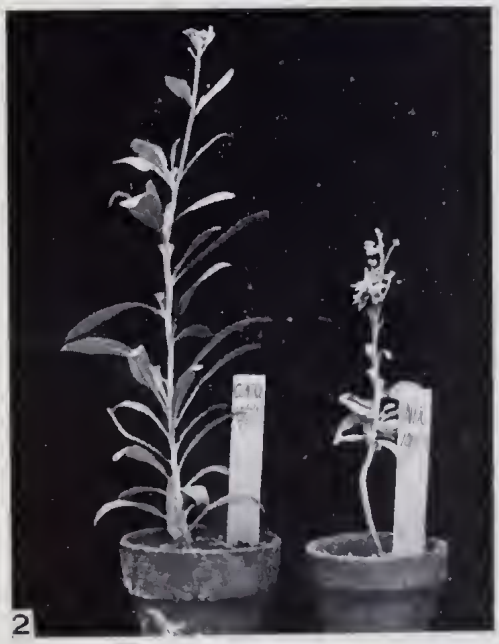
var., and cross inoculations were attempted. A pure culture study with full details was conducted and data on morphological, cultural, and physiological characters of the organism were secured.

Acknowledgments.

This work was carried out at the Botany School of the University of Melbourne, and thanks are expressed to Professor J. S. Turner for the facilities provided.

References.

1. Adam, D. B., and Pugsley, A. T.—Bacterial Plant Diseases in Victoria, *Jour. Dept. Agr. Vic.*, xxxii., pp. 304-311, 323, 1934.
2. Berridge, E. M.—The Influence of H-ion Concentration on the Growth of certain Bacterial Plant Parasites and Saprophytes, *Ann. Appl. Biol.*, xi., pp. 73-84, 1924.
3. Brown, N. A.—Some Bacterial Diseases of Lettuce, *Jour. Agr. Res.*, xiii., pp. 367-388, 1918.
4. Briosi, G., and Pavarino, L.—Una Malattia Batterica della "*Matthiola Annua* L." (*Bacterium matthiolae* n.sp.)—*Atti R. Accad. Lincei*, xxi., pp. 216-220, 1912.
5. ————. Bacteriosi della *Matthiola Annua* L. (*Bacterium matthiolae* n.sp.) *Atti Inst. Bot. Univ. Pavia*, xv., pp. 135-141, 1918.
6. Burkholder, W. H.—The Genus Phytomonas, *Phytopath.*, xx., pp. 1-23, 1930.
7. ————, Carbohydrate Fermentation by Certain Closely Related Species in the Genus Phytomonas, *Phytopath.*, xxii., pp. 699-707, 1932.
8. ————. A Bacterial Blight of Stocks Caused by *Phytomonas Syringae*, *Phytopath.*, xxviii., pp. 934-935, 1938.
9. ————. The Taxonomy and Nomenclature of the Phytopathogenic Bacteria, *Phytopath.*, xxix., pp. 128-136, 1939.
10. Clara, F. M.—A New Bacterial Leaf Disease of Tobacco in the Philippines, *Phytopath.*, xx., pp. 691-706, 1930.
11. ————. A Comparative Study of the Green Fluorescent Bacterial Plant Pathogens, *Cornell Univ. Agric. Exp. Sta. Mem.*, 159, 1934.
12. Cooley, L. M.—Black Rot of Stocks, *Phytopath.*, xxii., p. 270, 1932.
13. Faber von, F. C.—Über eine Bacterien Krankheit der Levkojen, *Arb. K. Anst. Land. u. Forstw.*, v., pp. 489-492, 1907.
14. Frazier, W. C., and Rupp, Ph.—Studies on Proteolytic Bacteria of Milk, *Jour. Bact.*, xvi., pp. 231-245, 1928.
15. Hensen, A.—The Detection of Ammonia Production by Bacteria in Agar Slants, *Jour. Bact.*, xix., pp. 223-229, 1930.
16. Johnson, J., Slagg, C. M., and Murwin, H. F.—Host Plants of *Bacterium Tabacum*, *Phytopath.*, xiv., pp. 175-180, 1924.
17. Kendrick, J. B.—A Seed-Borne Bacterial Disease of Garden Stocks—*Matthiola Incana*, *Phytopath.*, xxviii., p. 12, 1938.
18. Lacey, M. S.—Studies in Bacteriosis, *Ann. Appl. Biol.*, xviii., pp. 180-186, 1931.
19. ————. Studies in Bacteriosis, *Ann. Appl. Biol.*, xix., pp. 190-203, 1932.
20. Metha, M. M., and Berridge, E. B.—Studies in Bacteriosis, *Ann. Appl. Biol.*, xi., pp. 318-323., 1924.



(Page 203.)

10793/40.

21. Niederkorn, E.—Vergleichende Untersuchung Über die Verschiedenen Varietäten des *Bac. Pyocyaneus* und des *Bac. Fluorescens Liquefaciens, Centralbl. Bakt. Parasitenk.*, xvii., pp. 749-750, 1900.

22. Reid, J. J., Farrell, M. A., and Haley, D. E.—Bacterial Leaf Spot Diseases, *Science* N.S., lxxxix., pp. 566-567, 1939.

23. *Review of Applied Mycology*—Rassegna dei Casi Fitopatologici osservatinel 1937, xvii., p. 459, 1938.

24. Rudorf, G., and Job, M. M.—Noticias sobre una Bacteriosis en Cultivos de Alhelies "*Matthiola Incana*" Var. *Annua*, *Physis*, xi., pp. 122-127, 1932.

25. Ruzicka, S.—Experimentelle Studien über die Variabilität Wichtiger Character des *B. Pyocyaneus* und des *B. Fluorescens Liquefaciens*, *Centralb. Bakt. Parasitenk.*, xxiv., pp. 11-17, 1898.

26. Smith, E. F.—Effect of Desiccation, Bacteria in Relation to Plant Diseases, i., pp. 70-71, 1905.

27. ————. Effect of Direct Sunlight, Bacteria in Relation to Plant Diseases, i., pp. 71-72, 1905.

28. ————. The Dutch Disease of Wallflower, Bacteria in Relation to Plant Diseases, iii., p. 277, 1914.

29. White, H. E.—Bacterial Spot of Radish and Turnip, *Phytopath.*, xx., pp. 653-663, 1930.

30. Wilson, R. D.—A Bacterial Disease of Stock, *Jour. Austr. Inst. Agr. Sc.*, iv., pp. 212-215, 1938.

Explanation of Plate.

Plate VI.

Fig. 1.—A patch of diseased stocks with the healthy plants in the background.

Fig. 2.—Left: Healthy Control.
Right: Stock infected artificially by stem inoculation.

[Photos. M. Rothberg.]

10793 40.—14